

CLAIMS

We claim:

- 5 1. A method for selecting a primer, comprising:
 - a) providing:
 - i) a target nucleic acid having at least one accessible
site and at least one inaccessible site;
 - 10 ii) a plurality of extension primers, each of said
primers comprising a first region, wherein said first regions of said
plurality of primers differ in sequence from each other, and wherein said
plurality of primers comprise first regions that are complementary to
different portions of said target nucleic acid; and
 - 15 iii) a template-dependent nucleic acid extension agent;
 - b) exposing said plurality of extension primers and said extension
agent to said target nucleic acid under conditions wherein primers comprising first
regions that are complementary only to an inaccessible site in said target nucleic
acid are not extended by said extension agent, and wherein primers comprising
first regions that are complementary to at least one accessible site of said target
20 nucleic acid form an extension product;
 - c) selecting a primer complementary to at least one accessible site by
identifying a member of said plurality of primers that forms an extension product.
- 25 2. The method of Claim 1, wherein said target nucleic acid comprises DNA.
3. The method of Claim 1, wherein said target nucleic acid comprises RNA.
4. The method of Claim 1, wherein said plurality of primers further comprise
a second region, said second region located 5' of said first region.
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5. The method of Claim 4, wherein said second regions of said plurality of primers are identical in sequence to one another.

6. The method of Claim 5, further comprising providing:

5 i) first and second amplification primers, said first amplification primer complementary to at least a portion of said second regions of said plurality of extension primers and said second amplification primer capable of hybridizing to a sequence complementary to a first domain of said target nucleic acid; and

ii) an amplification agent;

10 and further comprising the step of treating said extension products with said first and second amplification primers and said amplification agents to produce amplification products prior to said selecting step.

7. The method of Claim 1, wherein said plurality of primers comprises at
15 least 10 different primers.

8. The method of Claim 1, wherein said plurality of primers comprises at least 100 different primers.

20 9. The method of Claim 1, wherein said plurality of primers comprises at least 1000 different primers.

10. The method of Claim 1, wherein said plurality of primers comprises a sufficient number of primers to encompass every sequence variation within said first
25 region.

11. The method of Claim 1, wherein said first region is six or more nucleotides in length.

30 12. The method of Claim 11, wherein said first region is six nucleotides in length.

13. The method of Claim 1, wherein said template-dependent nucleic acid extension agent comprises a polymerase.

5 14. The method of Claim 1, wherein said template-dependent nucleic acid extension agent comprises a reverse transcriptase.

15. A composition comprising an oligonucleotide, said oligonucleotide comprising a sequence of a first region of a primer selected using the method of Claim 1.

10 16. A method for identifying accessible sites on a target nucleic acid comprising:

a) providing:

15 i) a target nucleic acid having at least one accessible site and at least one inaccessible site;

20 ii) a plurality of extension primers, each of said primers comprising a first region, wherein said first regions of said plurality of primers differ in sequence from each other, and wherein said plurality of primers comprise first regions that are complementary to different portions of said target nucleic acid; and

iii) a template-dependent nucleic acid extension agent;

25 b) exposing said plurality of extension primers and said extension agent to said target nucleic acid under conditions wherein primers comprising first regions that are complementary only to an inaccessible site in said target nucleic acid are not extended by said extension agent, and wherein primers comprising first regions that are complementary to at least one accessible site of said target nucleic acid form an extension product that is complementary to said target nucleic acid adjacent to said accessible site;

30 c) determining at least a portion of the sequence of an extension product; and

d) identifying said accessible site by locating a region of said target nucleic acid adjacent to sequence that is complementary to said extension product.

17. The method of Claim 16, wherein said target nucleic acid comprises DNA.

18. The method of Claim 16, wherein said target nucleic acid comprises RNA.

19. The method of Claim 16, wherein said plurality of primers comprises at least 10 different primers.

20. The method of Claim 16, wherein said plurality of primers comprises at least 100 different primers.

21. The method of Claim 16, wherein said plurality of primers comprises at least 1000 different primers.

22. The method of Claim 16, wherein said plurality of primers comprises a sufficient number of primers to encompass every sequence variation within said first region.

23. The method of Claim 16, wherein said first region is six or more nucleotides in length.

24. The method of Claim 23, wherein said first region is six nucleotides in length.

25. The method of Claim 16, wherein said template-dependent nucleic acid extension agent comprises a polymerase.

26. The method of Claim 16, wherein said template-dependent nucleic acid extension agent comprises a reverse transcriptase.

27. A composition comprising an oligonucleotide, said oligonucleotide comprising a sequence complementary to an accessible site identified by the method of Claim 16.

28. A method of locating accessible sites on a target nucleic acid comprising:

a) providing:

i) a target nucleic acid having at least one accessible site and at least one inaccessible site;

ii) a plurality of extension primers, each of said primers comprising first region and second regions, wherein said first regions of said plurality of primers differ in sequence from each other, wherein said plurality of primers comprise first regions that are complementary to different portions of said target nucleic acid, and wherein said second region is located 5' of said first region;

iii) a template-dependent nucleic acid extension agent;

iv) an amplification agent; and

v) first and second amplification primers, said first amplification primer complementary to at least a portion of said second regions of said plurality of extension primers and said second amplification primer capable of hybridizing to a sequence complementary to a first domain of said target nucleic acid;

b) exposing said plurality of extension primers and said extension agent to said target nucleic acid under conditions wherein primers comprising first regions that are complementary only to an inaccessible site in said target nucleic acid are not extended by said extension agent, and wherein primers comprising first regions that are complementary to at least one accessible site of said target nucleic acid form an extension product;

c) treating said extension products with said amplification agent and said first and second amplification primers to generate one or more amplification products, said amplification products having a length, wherein said length of said

amplification products provides a distance of an accessible site on said target nucleic acid from said first domain of said target nucleic acid; and

d) determining a location of one or more accessible sites on said target nucleic acid using said distance.

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29. The method of Claim 28, wherein said using said distance comprises determining said size of one or more of said amplification products.

30. The method of Claim 28, wherein said target nucleic acid comprises DNA.

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31. The method of Claim 28, wherein said target nucleic acid comprises RNA.

32. The method of Claim 28, wherein said plurality of primers comprises at least 10 different primers.

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33. The method of Claim 28, wherein said plurality of primers comprises at least 100 different primers.

34. The method of Claim 28, wherein said plurality of primers comprises at least 1000 different primers.

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35. The method of Claim 28, wherein said plurality of primers comprises a sufficient number of primers to encompass every sequence variation within said first region.

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36. The method of Claim 28, wherein said first region is six or more nucleotides in length.

37. The method of Claim 36, wherein said first region is six nucleotides in length.

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38. The method of Claim 28, wherein said template-dependent nucleic acid extension agent comprises a polymerase.

39. The method of Claim 28, wherein said template-dependent nucleic acid extension agent comprises a reverse transcriptase.

40. The method of Claim 28, wherein said amplification agent comprises a polymerase.

41. The method of Claim 40, wherein said polymerase comprises a thermostable polymerase.

42. The method of Claim 28, wherein said treating said extension products with said amplification agent and said first and second amplification primers comprises a polymerase chain reaction.

43. A composition comprising an oligonucleotide, said oligonucleotide comprising a sequence complementary to an accessible site determined by the method of Claim 28.

44. A method comprising:

a. providing:

i. the oligonucleotide of Claim 27; and

ii. a target nucleic acid;

b. exposing said oligonucleotide to said target nucleic acid.

45. The method of Claim 44, wherein said target nucleic acid is present in a cell.

46. The method of Claim 44, wherein said cell is present in an animal.

47. The method of Claim 46, wherein said animal comprises a human.

48. A composition comprising an oligonucleotide, said oligonucleotide comprising a sequence selected from the group consisting of SEQ ID NOs:164-231, 236-
5 239, 241, 242, 244, 246-258, 260-269, 271-284, 286-302, 304-314, and 316-330.

49. The composition of Claim 48, wherein said oligonucleotide comprises a sequence selected from the group consisting of SEQ ID NOs:164-229.

10 50. A method for detecting the presence of an HIV target sequence comprising:

a. providing:

- i. a sample suspected of containing an HIV target sequence; and
- ii. an oligonucleotide selected from the group consisting of SEQ
15 ID NOs: 160-229;

b. exposing said sample to said oligonucleotide; and

c. detecting the presence or absence of said HIV target sequence in said sample.

20 51. The method of Claim 50, wherein said exposing step comprises conducting an invasive cleavage assay.